IN THE UNITED STATES PATENT AND TRADEMARK OFFICE
BEFORE THE BOARD OF PATENT APPEALS AND INTERFERENCES

In re Patent Application of

BIRCH, J. et al. Atty. Ref.: 4145-14

Serial No. 10/501,777 TC/A.U.: 1633

Filed: July 19, 2004 Examiner: Epps-Smith

For: GLUTAMINE-AUXOTHROPHIC HUMAN CELLS CAPABLE OF PRODUCING PROTEINS AND CAPABLE OF GROWING IN A GLUTAMINE-FREE MEDIUM

* *

Monday, July 12, 2010

Mail Stop Appeal Brief - Patents
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

APPEAL BRIEF

Sir:

Appellant hereby appeals the rejection of claims 32-41, in the Office Action dated September 28, 2009, and submits the present Appeal Brief pursuant to 37 CFR § 41.37. The application has been twice rejected.

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(1) REAL PARTY IN INTEREST

The real party in interest is LONZA BIOLOGICS PLC., 228 BATH ROAD, SLOUGH, BERKSHIRE, UNITED KINGDOM SL1 4DY, by way of an Assignment from the inventors, recorded in the U.S. Patent and Trademark Office on July 19, 2004, at Reel 016143, Frame 0960.

(2) RELATED APPEALS AND INTERFERENCES

The appellant, the appellant's legal representative, and the assignee are not aware of any related prior or pending appeals or interferences or judicial proceedings which will directly affect or be directly affected by or have a bearing on the Board's decision in this appeal.

(3) STATUS OF THE CLAIMS

Claims 32-41 are pending.

The application has been twice rejected.

The originally-filed claims 1-9 were amended, without prejudice, in a Preliminary Amendment filed July 19, 2004.

Claims 2 and 9 were canceled, claims 10-15 added, and claim 1 amended, without prejudice, in an Amendment filed February 21, 2006.

Claims 6, 12, 13, 14 and 15 were amended and claims 16-24 added, without prejudice, in a Supplemental Amendment filed March 26, 2006.

Claim 1 was amended, without prejudice, in an Amendment After Final Rejection filed August 25, 2006. The Amendment After Final Rejection filed August 25, 2006 was entered with the filing of a Request for Continued Examination (RCE) on September 26, 2006.

Claims 1 and 7 were amended, claims 8, 12, 13 and 16 were canceled and claims 25-27 were added, without prejudice, in a Response filed April 30, 2007.

Claims 17, 21, 23 and 27 were amended, claims 25 and 26 were canceled and claims 28-31 were added, without prejudice, in an Amendment After Final Rejection filed October 25, 2007. The Amendment After Final Rejection filed October 25, 2007

was entered with the filing of a Request for Continued Examination (RCE) on October 25, 2007.

Claims 1, 10, 14, 17, 18, 21, 22, 23, 28 and 29 were amended, claims 11, 30 and 31 were canceled, and claims 32-35 were added, without prejudice, in an Amendment filed July 16, 2008.

Claims 1, 3-7, 10, 14, 15, 17-24 and 27-29 were canceled, without prejudice, in an Amendment After Final Rejection filed July 2, 2009. The Amendment After Final Rejection filed July 2, 2009 was entered with the filing of a Request for Continued Examination (RCE) on July 15, 2009.

Claims 32, 33, 34 and 35 were amended, without prejudice, and claims 36-41 added, in a Supplemental Amendment filed August 17, 2009. The Supplemental Amendment filed August 17, 2009 has been entered. See Interview Summary October 5, 2009 and Examiner Interview Summary dated October 13, 2009.

A copy of all the rejected claims 32-41, i.e., the claims involved in the appeal, is attached as a Claims Appendix, pursuant to Rule 41.37(c)(1)(viii).

(4) STATUS OF THE AMENDMENTS

A response to the Office Action of September 28, 2009 has not been filed.

(5) SUMMARY OF CLAIMED SUBJECT MATTER

Pursuant to 37 CFR § 41.37(c)(1)(v), the following is a concise explanation of the subject matter defined in each of the independent claims (i.e., independent claim 32) involved in the appeal, which shall refer to the specification by page and line number, and to the drawing, if any, by reference characters.

Claim 32 is the only independent claims of the claims on appeal.

Independent claim 32 defines a method of increasing sialylation[1] and/or N-glycan charge[2] of a glycosylated protein expressed by a glutamine auxotrophic human cell[3] and of extending the viability of said cell[4], said method comprising transfecting a glutamine auxotrophic human cell with an exogenous DNA sequence encoding a glutamine synthetase to produce a transfected human cell[5] and culturing said transfected human cell in a glutamine-free media[6] such that said sialylation and/or N-glycan charge of said glycosylated protein is increased[7] and the viability of said cell is extended[8].

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[1] See for example, page 27, lines 4-5 of the specification.
[2] See for example, page 24, lines 5-14 of the specification.
[3] See for example, page 6, lines 13-30, page 11, lines 4-11 and the Examples of the specification.
[4] See for example, page 25, lines 3-4 of the specification.
[5] See for example, page 7, lines 14-25 and the Examples, such as Examples 6 and 7, of the specification
[6] See for example, Examples 6 and 7 of the specification.
[7] See for example, Example 9 and Table 9 of the specification.
[8] See for example, Example 10 of the specification.

Support for the details of dependent claim 33 may be found, for example, in originally-filed claims 1 and 7, and page 2, lines 31-32, and page 12, lines 18-20 of the specification.

Support for the details of dependent claims 34 and 35 may be found, for example, on page 7, line 27 through page 8, line 5 of the specification.

Support for the details of dependent claim 36 may be found, for example, on page 11, line 4 through page 12, line 4 of the specification.

Support for the details of dependent claim 37 may be found, for example, in originally-filed claims 3 and 4 and page 6, lines 15-17 of the specification.

Support for the details of dependent claim 38 may be found, for example, on page 6, lines 24-25 and the Examples of the specification as well as originally-filed claim 4.

Support for the details of dependent claim 39 may be found, for example, in originally-filed claim 5 and page 6, line 25 as well as the Examples of the specification.

Support for the details of dependent claims 40 and 41 may be found, for example, at page 1, line 4, page 8, lines 23-25 and the Examples of the specification.

(6) GROUNDS OF REJECTION TO BE REVIEWED ON APPEAL

The following ground of rejection are presented for review:

Whether the invention of claims 32-41 would have been obvious from the combined teachings of Wilson (WO 87/04462) or Bebbinton (U.S. Patent No. 5,891,693) "as evidenced by" Barsomian (U.S. Patent No. 5,238,821), in view of Brandt (U.S. Patent No. 6,395,484), Schneider (Journal of Biotechnology, Vol. 46, pages 161-185 (1996)), Gawlitzek (Biotechnology & Bioengineering, Vol. 68, No. 6, June 2000, pages 637-646) and Hermentin (U.S. Patent No. 6,096,555), as defined by 35 U.S.C. § 103.

(7) ARGUMENT

The methods of claims 32-41 would not have been obvious from the combined teachings of Wilson (WO 87/04462) or Bebbinton (U.S. Patent No. 5,891,693) "as evidenced by" Barsomian (U.S. Patent No. 5,238,821), in view of Brandt (U.S. Patent No. 6,395,484), Schneider (Journal of Biotechnology, Vol. 46, pages 161-185 (1996)), Gawlitzek (Biotechnology & Bioengineering, Vol. 68, No. 6, June 2000, pages 637-646) and Hermentin (U.S. Patent No. 6,096,555), and the rejection of claims 32-41 under 35 U.S.C. § 103 over the cited combination of art should be reversed. Consideration of the following in this regard is requested.

The claims define methods of increasing sialylation and/or N-glycan charge of a glycolylated protein expressed by a glutamine auxotrophic human cell. The appellants have discovered, and the application exemplifies, that the elevated rate of synthesis of a sialylated protein when a transfected cell according to the claimed invention is grown in a glutamine-free media. The Examples further demonstrate the reduced concentration of ammonia produced by a transfected cell according to the claimed invention in a glutamine-free media as compared to the concentration of ammonia a control cell not containing the second exogenous DNA sequence produces in media containing glutamine. The appellants have unexpectedly discovered that glutamine-auxotropic human cells are able to increase sialylation and/or N-glycan charge of a glycosylated protein expressed by the cell if the cell is

transfected with an exogenous DNA sequence encoding a glutamine synthetase and grown or cultured in a glutamine-free media. The cells of the disclosure reduce the concentration of ammonia in cell culture or media when grown in a glutamine-free media or culture which allows for a greater rate of protein synthesis and increased maximum product concentration (see for example, page 25, lines 3-7 of the specification) and increased degree of sialylation of the expressed glycosylated product (see for example, page 27, lines 2-5 of the specification).

There is no suggestion in the cited art or reasonable expectation from the cited art that a glutamine auxotrophic cell line, as required by the present claims, i.e., a cell line that by definition of the term auxotrophic is unable to synthesize glutamine, could grow in a glutamine-free media, as required by the present claims, i.e., grown in such a manner to increase sialylation and/or N-glycan charge and extend cell viability. There was no reasonable expectation from the cited art that a glutamine auxotrophic cell line, which has been transfected with a DNA sequence encoding a glutamine synthetase, could be grown in a glutamine free media, as required by the claims, such that sialylation and/or N-glycan charge is increased and cell viability is extended.

Further, the claimed invention is contrary to the suggestions of the cited art.

Specifically, the Examiner cites Gawlitzek (Biotechnology and Bioengineering, Vol. 68, No. 6, June 2000, pp 637-646) to allegedly

> "teach that in the culture of mammalian cells, the metabolite ammonium is produced as a by-product of glutamine metabolism and the thermal degradation of glutamine. Gawlitzek et al. further teaches that increased amounts of ammonium in cells leads to a decrease in terminal galactosylation and sialylation of TNFR-IgG. Thus, the reference provides a suggestion for increasing sialylation and/or N-glycan charge of a glycosylated protein in a cell without adding glutamine. [The reference] teach[es] that increasing the N-glycan of a glycosylated protein, i.e. increasing the ratio of glycosylation of a glycosylated protein, decides [sic, determines?] the activity of the protein." See pages 5-6 of the Office Action dated January 28, 2009, and page 7 of the Office Action of September 28, 2009 .

As stated by the Examiner, Gawlitzek teaches that

> "[a]mmonium is mainly a byproduct of glutamine metabolism and the thermal degradation of glutamine" See page 637 of Gawlitzek, right column.

As also suggested by the Examiner, Gawlitzek teaches that

> "[a]s ammonium increased from 1 to 5 mM, a concomitant decrease of up to 40% was observed in terminal galactosylation and sialylation of the [TNFR-IgG] molecule." See Abstract of Gawlitzek.

The Examiner's conclusion drawn from, and reliance on, the reference however is contrary to Gawlitzek. Specifically, the Examiner concludes that Gawlitzek allegedly provides a suggestion for increasing sialylation and/or N-glycan charge of a glycosylated protein in a cell by limiting or eliminating glutamine. See for example, page 11 of the Office Action dated September 28, 2009.

While Gawlitzek may mention in the introduction (last sentence, page 637 – with reference to Schneider et a. J Biotechnol. 1996), that numerous studies reported negative effects of elevated ammonium concentrations on cell growth and productivity of different cell lines, Gawlitzek is understood to have observed "no detrimental effect of high ammonium on cell growth or viability" and "cell-specific productivity of TNFR-IgG was similar in all cases" (page 641, first paragraph, left column; Figure 1). In other words, Gawlitzek was unable to confirm the findings described by others such as Schneider et al. Gawlitzek neither teaches nor suggests a method to extend cell viability or a method to increase sialylation by way of transfecting a cell line with a GS gene, as claimed.

In fact, the Discussion of Gawlitzek taken with the results of Figure 7 of Gawlitzek, for example, indicate that limiting or eliminating glutamine in the model CHO cell of Gawlitzek will not effect terminal galactosylation and sialylation.

More specifically, Gawlitzek concluded as follows:

> "The results presented here strongly suggest that ammonium inhibits galactosylation and sialylation of TNFR-IgG N-glycans by pH-regulated mechanisms. We hypothesize that ammonium decreases α2,3-sialyltransferase and β1,4-galactosyltransferase activities by increasing the pH of the *trans*-Golgi compartment." See page 644, right column, first two sentences of the last paragraph, of Gawlitzek.

Gawlitzek concludes therefore that ammonium decreases transferase enzyme activity related to terminal galactosylation and sialylation due to an increase in pH.

The following reproduction of Figure 8 of Gawlitzek (see page 644 of the reference) demonstrates that α2,3-sialyltransferase and β1,4-galactosyltransferase activities are pH dependent.

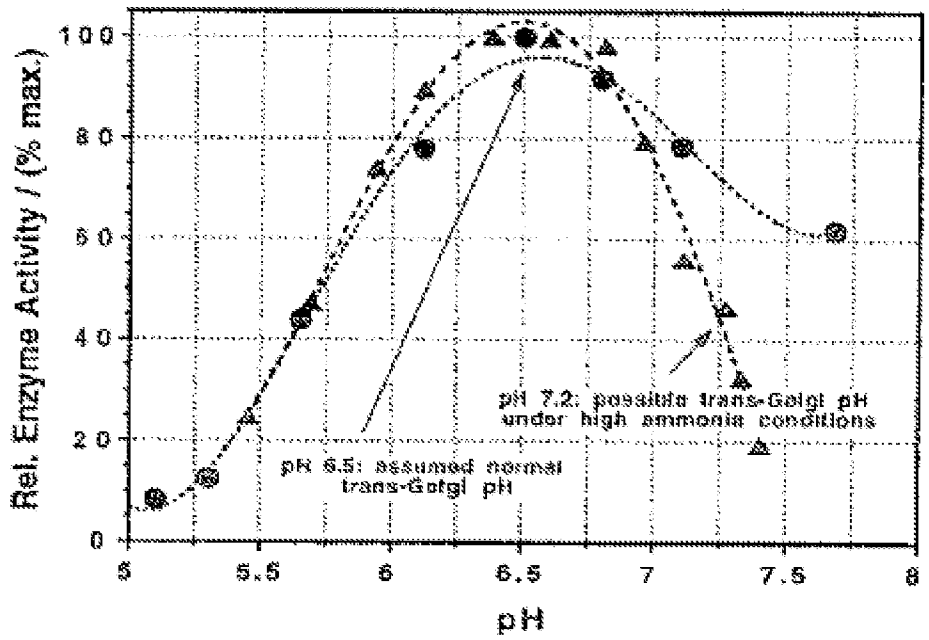
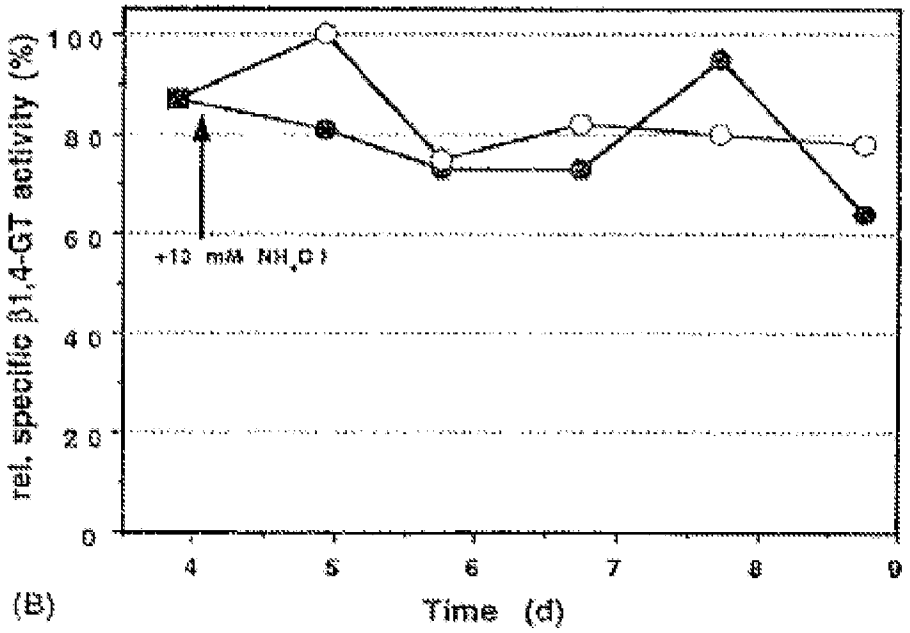


Figure 8. The pH profile of CHO α2,3-ST (▲) and β1,4-GT (●) activity.

The following reproduction of Figure 7 of Gawlitzek however demonstrates that similar enzyme activities were found when comparing cells cultivated under low and high ammonium concentrations (see also the description of Figure 7 on page 643 of Gawlitzek):



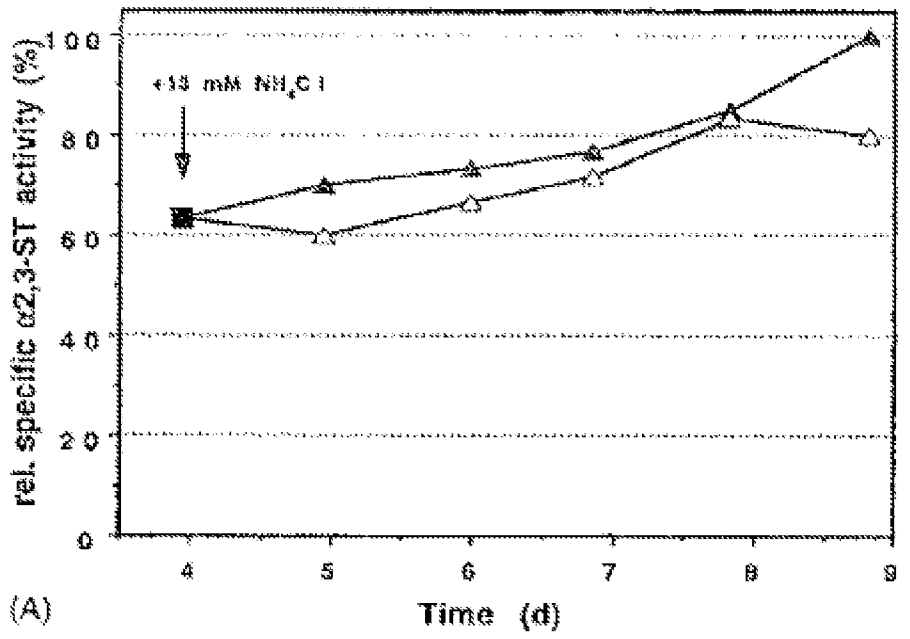


Figure 7. Enzyme activities of β1,4-galactosyltransferase and α2,3-sialyltransferase in CHO cells cultivated under control (−Gln) and high-ammonium (−Gln/+13 m*M* NH_4Cl) conditions. On day 4, 13 m*M* NH_4Cl was added after the enzyme samples were taken.

Gawlitzek also demonstrates with the following Figure 6 that similar mRNA levels of the enzymes were found when comparing the CHO cells cultivated under low or high ammonium concentrations.

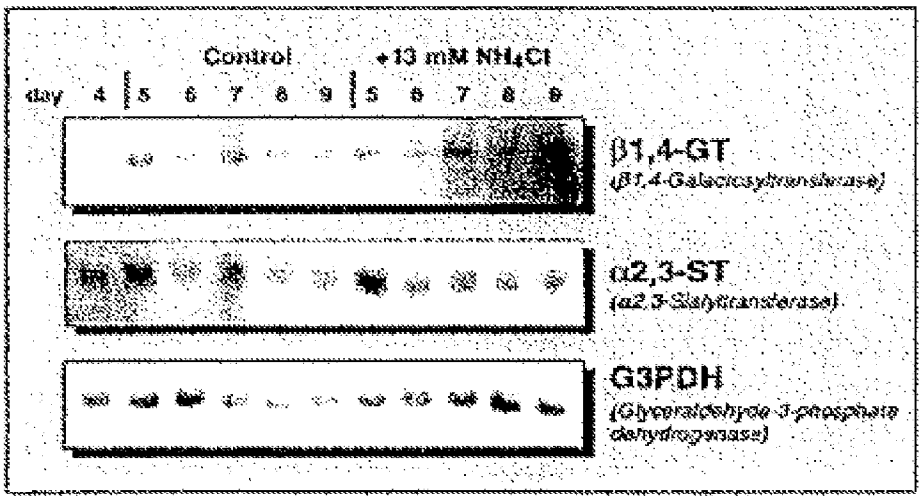


Figure 6. Northern blot analysis of β1,4-galactosyltransferase and α2,3-sialyltransferase mRNA in CHO cells cultivated under control (−Gln) and high-ammonium (−Gln/+13 m*M* NH_4Cl) conditions (see Materials and Methods for details). mRNA levels of glycosyltransferases compared with those of the housekeeping enzyme, glyceraldehyde-3-phosphate dehydrogenase.

Gawlitzek therefore teaches that while ammonium concentrations may effect terminal galactosylation and sialylation of TNFR-IgG in CHO cells and while ammonium concentrations may be decreased by eliminating glutamine from the culture media of the CHO cells of Gawlitzek (see Figure 2 of Gawlitzek), the reduction of ammonium concentrations provided by elimination of glutamine from the

culture media of the CHO cells of Gawlitzek would not effect terminal galactosylation and sialylation of TNFR-IgG in CHO cells.

Gawlitzek therefore teaches away from a method of the claimed invention.

As Gawlitzek is understood to be the only reference cited by the Examiner to allegedly teach or suggest a connection between glutamine concentration and terminal galactosylation and sialylation, the Section 103 rejection should be withdrawn in view of the above demonstration that Gawlitzek teaches that any effect of ammonium on terminal galactosylation and sialylation of TNFR-IgG in CHO cells is related to reduced activity of transferase enzymes at the increased pH caused by the presence of ammonium and that similar transferase enzyme expression and activities are present in CHO cells in the presence and absence of glutamine in the culture media.

None of the remaining cited references (i.e., Wilson (WO 87/04462), Bebbington (U.S. Patent No. 5,891,693), Barsomian (U.S. Patent No. 5,238,821), Brandt (U.S. Patent No. 6,395,484) and Hermitin (U.S. Patent No. 6,096,555)) cure these deficiencies of Gawlitzek. The cited combination of art fails to teach or suggest the claimed invention.

Specifically, Wilson teaches a DNA sequence which encodes the amino acid sequence of CHO glutamine synthetase as well as methods of making and using similar sequences. Wilson fails to teach or suggest any relationship however

between transfection of a glutamine auxotrophic human cell with an endogenous DNA sequence encoding a glutamine synthetase and production of glycosylated proteins with an increased sialylation and/or N-glycan charge, as claimed.

Bebbington teaches production of a lymphoid cell line which is glutamine independent and the advantage of growing the transformed lymphoid cell line on a medium containing glutamine followed by growth where glutamine is progressively depleted. See column 1, lines 28-67 of Bebbington, for example. Bebbington does not describe glycosylation patterns of proteins produced from the cell lines or any effect of transformation and growth conditions on same. Bebbington fails to teach or suggest any relationship however between transfection of a glutamine auxotrophic human cell with an enogenous DNA sequence encoding a glutamine synthetase and production of glycosylated proteins with an increased sialylation and/or N-glycan charge, as claimed.

The combined teachings of Gawlitzek and Wilson or Gawlitzek and Bebbington would not have made the claimed invention obvious.

Barsomian provides enzymes useful to

> "completely deglycosylate an asparagine-glycoprotein or glycopeptides. This is useful for protein sequencing, isoelectric focusing, peptide mapping, and in the two dimensional electrophoresis of glycopeptides and glycoproteins." See column 3, lines 12-17 of Barsomian.

Barsomian does not describe glycosylation patterns of proteins produced from cell lines or any effect of transformation and growth conditions on same. Barsomian fails to teach or suggest any relationship between transfection of a glutamine auxotrophic human cell with an endogenous DNA sequence encoding a glutamine synthetase and production of glycosylated proteins with an increased sialylation and/or N-glycan charge, as claimed.

The combined teachings of Gawlitzek, Barsomian and Wilson or Gawlitzek, Barsomian and Bebbington would not have made the claimed invention obvious.

Hermentin provides methods for characterizing the glycolysation of glycoproteins and the in vitro determination of the bio-availability of glycoproteins. Glycoproteins of the examples of Hermentin appear to be produced in CHO and BHK cells. Specific descriptions of the production conditions of the glycoproteins of Hermentin do not appear to be provided.

Hermentin does not any effect of transformation and growth conditions on glycosylation. Hermentin fails to teach or suggest any relationship between transfection of a glutamine auxotrophic human cell with an endogenous DNA sequence encoding a glutamine synthetase and production of glycosylated proteins with an increased sialylation and/or N-glycan charge, as claimed.

The combined teachings of Gawlitzek, Barsomian, Hermentin and Wilson or Gawlitzek, Barsomian, Hermentin and Bebbington would not have made the claimed invention obvious.

Brandt provides a method of identification of human cell lines for the production of human proteins by endogenous gene activation. The method of Brandt requires transfecting cells with a DNA construct comprising specific flanking sequences, positive selection marker, optional negative selection marker, optional amplification gene, and a heterologous expression control sequence which is active in human cells. See column 4, lines 18-25 of Brandt. Brandt teaches that producing recombinant target proteins with the "correct glycosylation" is a goal. See column 3, line 24 of Brandt. The examples of Brandt all appear to teach growth of transfected cells (Namalwa cells, HT1080 cells and HeLa S3 cells) in the presence of glutamine and fetal calf serum. See Example 5 of Brandt.

Brandt does not describe effects of transformation and growth conditions on glycosylation of proteins. Brandt fails to teach or suggest any relationship between transfection of a glutamine auxotrophic human cell with an endogenous DNA sequence encoding a glutamine synthetase and production of glycosylated proteins with an increased sialylation and/or N-glycan charge, as claimed.

The combined teachings of Gawlitzek, Barsomian, Hermentin, Brandt and Wilson or Gawlitzek, Barsomian, Hermentin, Brandt and Bebbington would not have made the claimed invention obvious.

The claims are submitted to be patentable over the combinations of Wilson (WO 87/04462) or Bebbington (U.S. Patent No. 5,891,693) "as evidenced by" Barsomian (U.S. Patent No. 5,238,821) in view of Brandt (U.S. Patent No. 6,395,484) and Gawlitzek (Biotechnology and Bioengineering, Vol. 68, No. 6, June 2000, pp 637-646) and Hermitin (U.S. Patent No. 6,096,555).

In an Interview Summary dated March 9, 2010, the Examiner further states that

> "The method produced by the combination of the cited references clearly suggests a method of increasing sialylation and/or N-glycan charge of a glycosylated protein. Absent evidence to the contrary, the method suggested by the combination of the cited references would also produce the "intended use" limitation set forth in the preamble of the claim."

The Board is urged to appreciate that the claims define methods. The Examiner's reference to the appellants stated "intended use" as a basis for rejecting the claimed methods as allegedly having been obvious is inappropriate and can not be sustained. A concern over a recited "intended use" limitation in a preamble is more usually related to claims defining old products which are defined as being "for use" in some alleged new manner. The presently claimed invention however defines

methods which provide unexpected benefits in increasing sialylation and/or N-glycan charge of a glycosylated protein and in allowing for a greater rate of protein synthesis and increased maximum product concentration. These results would not have been expected from the cited combination of art. The claimed invention requires, as a result of the claimed method, the increase in sialylation and/or N-glycan charge of the glycosylated protein and extension of the viability of the cell of the claim.

The Examiner's reference to "intended use" further suggests that the Examiner believes the cells of the claimed methods are being claimed in *per se* and as such the result of that use would allegedly be inherent to the culturing of the cells. The Examiner's reliance on an alleged or uncertain characteristic of the cited art (i.e., increase in sialylation and/or N-glycan charge of the glycosylated protein and extension of the viability of the cell of the claim) can not be the basis of a *prima facie* case of obviousness, especially in the context of a method claim.

Specifically, the Court has explained in In re Rijckaert, 28 USPQ2d 1955, 1957 (Fed. Cir. 1993), for example, the following with regard to inherency and obviousness:

> The mere fact that a certain thing may result from a given set of circumstances is not sufficient [to establish inherency.]" In re Oelrich , 666 F.2d 578, 581-82, 212 USPQ 323, 326 (CCPA 1981) (citations omitted) (emphasis added). "That which may be inherent is not necessarily known. Obviousness cannot be predicated on what is unknown." In re Spormann, 363 F.2d 444, 448, 150 USPQ 449, 452 (CCPA 1966). Such a retrospective

> view of inherency is not a substitute for some teaching or suggestion supporting an obviousness rejection. See In re Newell, 891 F.2d 899, 901, 13 USPQ2d 1248, 1250 (Fed.Cir. 1989).

For reasons including those set forth above and of record, the Board is respectfully requested to reverse the Section 103 rejection of claims 32-41over the combination of Wilson (WO 87/04462) or Bebbinton (U.S. Patent No. 5,891,693) "as evidenced by" Barsomian (U.S. Patent No. 5,238,821), in view of Brandt (U.S. Patent No. 6,395,484), Schneider (Journal of Biotechnology, Vol. 46, pages 161-185 (1996)), Gawlitzek (Biotechnology & Bioengineering, Vol. 68, No. 6, June 2000, pages 637-646) and Hermentin (U.S. Patent No. 6,096,555).

Reversal of the 35 U.S.C. § 103, rejection of claims 32-41 is requested.

The claims are submitted to be in condition for allowance and Reversal of the final rejection is requested.

Respectfully submitted,

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(8) CLAIMS APPENDIX

32. A method of increasing sialylation and/or N-glycan charge of a glycosylated protein expressed by a glutamine auxotrophic human cell and of extending the viability of said cell, said method comprising transfecting a glutamine auxotrophic human cell with an exogenous DNA sequence encoding a glutamine synthetase to produce a transfected human cell and culturing said transfected human cell in a glutamine-free media such that said sialylation and/or N-glycan charge of said glycosylated protein is increased and the viability of said cell is extended.

33. The method of claim 32 wherein said glycosylated protein is encoded by an exogenous DNA sequence and is recovered from the culture of said transfected human cell.

34. The method according to claim 32, wherein said transfected human cell is further transfected with an amplifiable gene encoding an enzyme, wherein said enzyme is dihydrofolate reductase (DHFR), adenosine deaminase, asparagine synthetase, aspartate transcarbamylase, metallothionein-1, ornithine decarboxylase, P-glycoprotein, ribonucleotide reductase, thymidine kinase or xanthine-guanine phosphoribosyl transferase.

35. The method according to claim 33, wherein said transfected human cell is further transfected with an amplifiable gene encoding an enzyme, wherein said

enzyme is dihydrofolate reductase (DHFR), adenosine deaminase, asparagine synthetase, aspartate transcarbamylase, metallothionein-1, ornithine decarboxylase, P-glycoprotein, ribonucleotide reductase, thymidine kinase or xanthine-guanine phosphoribosyl transferase.

36. Method of claim 32 wherein the transfected cell is anchorage-independent and capable of growing in suspension in serum-free, glutamine-free medium.

37. Method of claim 32 wherein the glutamine-auxotrophic human cell is an immortalized glutamine-auxotrophic human cell.

38. Method of claim 37 wherein the immortalized glutamine-auxotrophic human cell is a human fibrosarcoma cell.

39. The method of claim 38, wherein the human fibrosarcoma cell is a HT1080 cell line.

40. Method of claim 32 wherein the glycosylated protein is erythropoietin.

41. The method of claim 40, wherein the erythropoietin is human erythropoietin.

(9) EVIDENCE APPENDIX

Attached:

NONE

(10) RELATED PROCEEDINGS APPENDIX

Attached:

NONE